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ORIGINAL ARTICLE

A new sensor based on glassy carbon electrode modified with nanocomposite for simultaneous determination of acetaminophen, ascorbic acid and uric acid

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Abstract A chemically-modified electrode has been constructed based on a single walled carbon nanotube/chitosan/room temperature ionic liquid nanocomposite modified glassy carbon electrode (SWCNTs–CHIT–RTIL/GCE). It was demonstrated that this sensor could be used for simultaneous determination of acetaminophen (ACT), uric acid (URI) and ascorbic acid (ASC). The measurements were carried out by application of differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometry (CA) methods. Electrochemical studies suggested that the RTIL and SWCNTs provided a synergistic augmentation that can increase current responses by improvement of electron transfers of these compounds on the electrode surface. The presence of the CHIT in the modified electrode can enhance the repeatability of the sensor by its antifouling effect. The modified electrode showed electrochemical responses with high sensitivity for ACT, URI and ASC determination, which makes it a suitable sensor for simultaneous sub-μmol L^{-1} detection of ACT, URI and ASC in aqueous solutions. The analytical performance of this sensor has been evaluated for detection of ACT, URI and ASC in human serum and urine with satisfactory results.

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1. Introduction

Carbon nanotubes (CNTs) are new kinds of porous nanostructured carbon materials, possessing properties such as high electrical conductivity, high surface area, chemical stability and significant mechanical strength (Yakobson and Smalley, 1997;

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Inagaki et al., 2004). CNTs can be used to promote electron transfer reactions when they are used as electrode materials in electrochemical devices (Yin et al., 2005; Yang et al., 2005).

Room temperature ionic liquids (RTILs) have been extensively used as a material for preparation of the modified electrode because of their interesting properties such as low toxicity, wide electrochemical potential window, high ionic conductivity and increasing the sensitivity of response (Zhao et al., 2004, 2007; Lu et al., 2006; Du et al., 2007).

A wide range of polymer and inorganic mesoporous metal oxides have been combined to form nanocomposite materials with unique mechanical, electrical, magnetic and adhesive properties (Gangopadhyay and De, 2000; Chiang and Whang, 2003). Chitosan (CHIT) as a natural polymer is biocompatible, biodegradable, nontoxic, highly hydrophilic with high mechanical strength, low cost, chemical inertness, and good adhesion properties that exhibit an excellent membrane-forming ability. Because chitosan–CNTs can form stable complexes through noncovalent binding, the stability of CNTs in aqueous chitosan solution was greatly improved. Thus, CNTs could be uniformly distributed in the chitosan film (Huang et al., 2002; Wang et al., 2002; Zhang and Zheng, 2008; Babaei et al., 2010a,b, 2011a,b).

Acetaminophen (ACT) is an important medicine, extensively used both in a pure form and in pharmaceutical preparations. It is mainly used as an alternative to aspirin as an analgesic and antipyretic agent that lacks the disadvantageous secondary effects of the salicylates on the gastric mucosa. It is often self-prescribed, without medical control, for relief of moderate pain, fever, lumbar pain, migraine or even non-specific indications. It has been reported to be a useful drug in osteoarthritis therapy. However an overdose of ACT can result in the accumulation of toxic metabolites that may cause severe and sometimes fatal hepatoxicity (Mugford and Tarloff, 1997). It can also cause liver disorders, skin rashes and inflammation of the pancreas (Prabakar and Narayanan, 2007). A number of analytical techniques such as spectrophotometry (Ayora Canada et al., 2000), spectrofluorometry (Vilchez et al., 1995), voltammetry (Babaei et al., 2010a,b, 2011a,b), HPLC (Ravisankar et al., 1998), colorimetry (Knochen et al., 2003) and Fourier transform infra red spectrometry (Ramos et al., 1998), have been proposed for the determination of ACT in pharmaceutical formulations and biological samples.

Uric acid (URI) is the primary end product of purine metabolism, and considered as a species of great importance in human diagnosis. The typical concentration of URI in blood is in the range of 120–450 μmol L^{-1}. An abnormal concentration of URI can indicate the presence of one of numerous diseases and/or physiological disorders. An elevated concentration of URI is observed in patients suffering from diseases such as gout and hyperuricemia (Dutt and Mottola, 1974; Zen et al., 1998). Because of its clinical relevance, it is crucial to develop simple and rapid methods for URI determination in routine analysis. Various methods have been used to accomplish this, such as enzyme-based systems (Zhao et al., 2009), fluorescence (Galban et al., 2001), chemiluminescence (Yang and Zhang, 2010), capillary electrophoresis (Zhao et al., 2008), liquid chromatography (Perelló et al., 2005) and voltammetry (Inada et al., 2009). A major problem for the electrochemical detection of URI is interfering species such as ACT, which is oxidized at a similar potential to that of URI making direct determination of ACT or URI unreliable.

Ascorbic acid (ASC) an essential nutrient can be found mainly in fruits and vegetables. The body requires ASC to form collagen protein to maintain bones, blood vessels, and skin. Due to its antioxidant and pH regulator properties, this vitamin is present or added to a wide variety of food products and pharmaceuticals. Ascorbic acid is easily oxidized chemically and electrochemically to L-dehydroascorbic acid (Sabzi and Pournaghi-Azar, 2005). ASC is unstable, undergoing oxidation, especially in aerobic conditions, alkaline media, and at exposure to light (Zeng et al., 2005). Ascorbic acid is widely found in association with various biologically and pharmacologically active substances, including acetaminophen (Săndulescu et al., 2000) in various pharmaceutical products as well as in biological fluids (Săndulescu et al., 2000; Zhang et al., 2001; Arvand et al., 2003; Ramesh and Sampath, 2004). An overdose amount of ASC in some people may lead to diarrhea, nausea, skin irritation, burning upon urination, and depletion of copper. In addition ASC can cause adverse reactions when taken with some drugs. Therefore determination of ASC in the presence of some popular medicines like ACT is of major interest (Săndulescu et al., 2000). Several available methods have been reported for the determination of ascorbic acid in pharmaceutical preparations, biological fluids, food and beverages. The methods are: fluorimetry (Yang et al., 2001), HPLC (Kand'ár and Záková, 2008), spectrophotometry (Salkić and Kubiček, 2008) and voltammetry (Satheesh Babu et al., 2010).

The advantages of an electrochemical technique for the determinations of ACT, URI and ASC are high sensitivity, low cost, and rapid measurement time. In this work we outline the use of a single-walled carbon nanotube/chitosan/room temperature ionic liquid nanocomposite modified glassy carbon electrode (SWCNTs–CHIT–RTIL/GCE) as a sensor for this purpose. To the best of our knowledge there has been no report of the use of an electrochemical sensor for the simultaneous determination of ACT, URI and ASC compounds. In addition, the analytical performance of this sensor for the determination of ACT, URI and ASC in human serum and human urine samples was evaluated with satisfactory results.

2. Experimental

2.1. Reagents

All chemicals were analytical grade and used as received. ACT, URI and ASC were obtained from Merck chemical company. Chitosan (MW 1.0–3.0 × 10^5) and Single-walled carbon nanotubes (SWCNTs) were purchased from Acros and Sigma companies, respectively. The purity of SWCNTs was 90% with a surface specific area of 480 m^2 g^{-1}, diameter of 1–2 nm and length of 0.5–2 μm.

1-Ethyl-3-methyl-imidazolium tetra-fluoro borat (EMIM·BF_4) was obtained from Merck chemical company. All ACT, URI and ASC solutions were prepared by diluting the stock standard solutions using 0.1 mol L^{-1} phosphate buffer (pH 7). 0.1 mol L^{-1} Phosphate buffer solution (PBS) was prepared by dissolving appropriate amounts of sodium hydrogen phosphate and sodium dihydrogen phosphate in a 250 mL volumetric flask. pH of solution was adjusted to an appropriate value by addition of 7.5 mol L^{-1} sodium hydroxide solution. Electrochemical experiments were carried out on ACT, URI and ASC in 0.1 mol L^{-1} PBS at pH 7.

Fresh human serum samples were obtained from the Razi Institute of Vaccine and Serum Company (Tehran, Iran). The serum and urine samples were filtered and diluted 40 times with 0.1 mol L^{-1} PBS of pH 7 before spiking with ACT, URI and ASC.

2.2. Instrumentation

All the voltammetric measurements were carried out using a nanocomposite modified glassy carbon electrode (SWCNTs–CHIT–RTIL/GCE) as a working electrode, Ag/AgCl/3 mol L^{-1} KCl as a reference electrode and platinum wire as an auxiliary electrode. DPV, CV and CA experiments were carried out using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm Switzerland). All of the potential were measured with respect to the reference electrode. pH measurements were performed with a Metrohm 744 pH meter using a combination glass electrode.

2.3. Modification of the electrodes

A glassy carbon electrode (GCE, 2 mm diameter, Metrohm) was polished with 0.3 and 0.05 μm alumina slurry and rinsed thoroughly with triply distilled water. The GC electrode was cleaned by ultrasonic agitation for 5 min in ethanol and then distilled water, individually. The electrode was then dried with nitrogen. To obtain a good quality SWCNTs–CHIT–RTIL/GC modified electrode, the concentrations and mass ratios of CHIT, SWCNTs and RTIL ([EMIM][BF4]) in the mixture were optimized in control experiments. A stock solution of 0.50 wt.% CHIT solution was prepared by dissolving 5 mg of CHIT in 1 mL of 1% acetic acid solution and the pH of the solution was adjusted to 5.0 with concentrated NaOH. With the aid of ultrasonic agitation for 30 min, 2.0 mg SWCNTs were dissolved in 1 mL of 0.5 wt.% CHIT and resulted in a homogeneous black SWCNTs–CHIT solution. Typically, a homogeneous solution containing 0.5 wt.% CHIT, 2.0 mg mL^{-1} SWCNTs and 5% (VRTIL/Vtotal) RTIL ([EMIM][BF4]) was obtained. 20 μL of SWCNTs–CHIT–RTIL solution was placed on the GC electrode surface and dried at room temperature to obtain the SWCNTs–CHIT–RTIL/GCE. The fabricated SWCNTs–CHIT–RTIL/GCE was placed in the electrochemical cell containing 0.1 mol L^{-1} PBS and several cycles in the potential window from −0.3 to 0.7 V were applied using the CV method to obtain stable responses.

2.4. General procedure

Ten mL solutions containing appropriate amounts of ACT, URI and ASC in 0.1 mol L^{-1} PBS at pH 7 were transferred into the voltammetric cell. The voltammograms were recorded by applying positive-going potential from −0.3 to 0.6 V. The voltammograms showed anodic peaks around 0.32, 0.20 and −0.09 V corresponding to ACT, URI and ASC compounds of which their peak currents were proportional to their concentrations in solutions. The calibration curves were obtained by plotting anodic peak currents of ACT, URI and ASC versus the corresponding concentrations. All experiments were carried out under an open circuit condition.

After each measurement, the SWCNTs–CHIT–RTIL/GCE was regenerated by thoroughly washing the electrode with triply distilled water and then 5% sodium hydroxide solution, consecutively. Finally the electrode was rinsed carefully with distilled water to remove all adsorbates from the electrode surface and to provide a fresh surface for the next experiments.

3. Results and discussion

3.1. Characterization of the SWCNTs–CHIT–RTIL/GCE

The SWCNTs–CHIT–RTIL/GC modified electrode was characterized by electrochemical methods. $K_3Fe(CN)_6$ exhibited a pair of quite reversible redox peaks at a bare GC electrode. At the modified electrode, a pair of higher and reversible redox peaks could still be observed. On the other hand, under the same conditions, the anodic peak of $K_3Fe(CN)_6$ at both the GC and SWCNTs–CHIT–RTIL/GC electrodes increased in proportion to the square root of the scan rate. It was found that in both cases the electrode process was diffusion controlled. The regression equations for the 4 mmol L^{-1} $K_3Fe(CN)^6$ were:

$$\mathrm{Ipa}(\mu\mathrm{A}) = 91.6v^{1/2}(\mathrm{V\,s^{-1}})^{1/2} + 4.3 \quad \text{GCE}$$

$$\mathrm{Ipa}(\mu\mathrm{A}) = 696.3v^{1/2}(\mathrm{V\,s^{-1}})^{1/2} + 5.1 \quad \text{SWCNTs–CHIT–RTIL/GCE}$$

***A reversible system should satisfy the Randles–Sevcik equation:

$$I_P = 2.99 \times 10^5 \alpha^{1/2} n^{3/2} A C_0 D_R^{1/2} v^{1/2}$$

According to the ratio of the slopes of the two lines, the apparent area of the SWCNTs–CHIT–RTIL/GC modified electrode was about 7.6 times greater than that of the GC electrode.

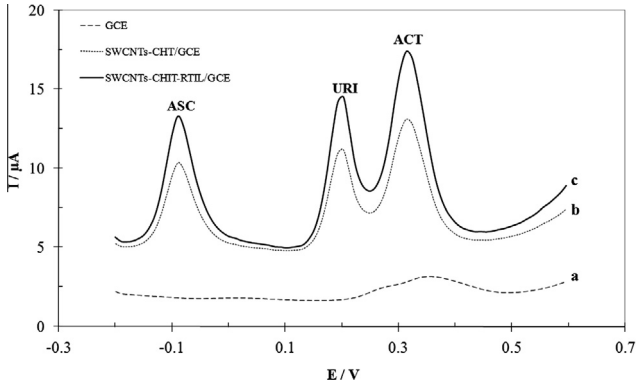

Figure 1 Differential pulse voltammograms of 30 μmol L^{-1} ACT, 40 μmol L^{-1} URI and 70 μmol L^{-1} ASC at (a) GC, (b) SWCNTs–CHT/GCE and (c) SWCNTs–CHIT–RTIL/GCE in 0.1 mol L^{-1} phosphate buffer solution (pH 7). Other conditions: Open circuit, tacc = 75 s, pulse amplitude = 50 mV, scan rate = 10 mV s^{-1}, interval time 0.5 s, modulation time = 0.2 s and step potential = 5 mV.

3.2. Electrooxidation behavior of ACT, URI and ASC on SWCNTs–CHIT–RTIL/GCE

The differential pulse voltammograms recorded for acetaminophen, uric acid and ascorbic acid from bare GCE, SWCNTs–CHIT/GCE and SWCNTs–CHIT–RTIL/GCE are shown in Fig. 1. Fig. 1(a) shows the voltammogram from a bare GCE of 30 μmol L^{-1} ACT, 40 μmol L^{-1} URI and 70 μmol L^{-1} ASC in PBS (pH of 7). Fig. 1(b) displays the voltammogram of ACT, URI and ASC from a SWCNTs–CHT/GCE and Fig. 1(c) displays the voltammogram of ACT, URI and ASC from a SWCNTs–CHIT–RTIL/GCE under the same conditions as for Fig. 1(a). As can be seen the presence of SWCNTs and RTIL appears to facilitate the electron transfer between electrode and the analytes causing enhancements in the corresponding electrochemical oxidation peak currents. In addition, the presence of RTIL and CHIT lead to the oxidation peak of URI shifts to less positive potentials and leads to more current peak separation between URI and ACT.

The effect of potential scan rate on the oxidation responses of ACT, URI and ASC was investigated in the 10–120 mV s^{-1} range of scan rate (not shown). Linear relationships between the anodic peak currents and scan rate were found for ACT, URI and ASC as follows:

$$\mathrm{Ipa}(\mu\mathrm{A}) = 0.070v(\mathrm{mV}\ s^{-1}) + 1.900 \quad \text{ACT}$$

$$\mathrm{Ipa}(\mu\mathrm{A}) = 0.091v(\mathrm{mV}\ s^{-1}) + 1.862 \quad \text{URI}$$

$$\mathrm{Ipa}(\mu\mathrm{A}) = 0.087v(\mathrm{mV}\ s^{-1}) + 3.460 \quad \text{ASC}$$

The linear relationship between peak currents and scan rates, suggesting that the redox reactions of ACT, URI and ASC compounds at SWCNTs–CHIT–RTIL/GCE, are adsorption-controlled processes.

At sweep rates higher than 100 mV s^{-1} peak separations (ΔEp) begin to increase, indicating the limitation due to charge transfer kinetics. Based on the Laviron theory the electron transfer rate constant (ks) and charge transfer coefficient (α) can be determined by measuring the variation of ΔEp vs. log scan rate. The slope of the ΔEp vs. log($\vee$), was about, −137.9 mV. Using the equation of:

$$Ep = K - 2.3030(RT/\alpha nF)\log(v)$$

By considering two electrons transferred for ACT, charge transfer coefficient (α) of 0.493 was obtained. Introducing α value in the following equation, an apparent surface electron transfer rate constant, ks = 2.07 s^{-1}, was estimated.

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nF\upsilon}\right) - \alpha(1-\alpha) \times \frac{nFE}{2.3RT}$$

The large value of electron transfer rate constant indicates the high ability of SWCNTs–CHIT–RTIL for promoting electron between ACT and the electrode surface.

3.3. Effects of supporting electrolyte and solution pH

The oxidation peak current of ACT, URI and ASC at SWCNTs–CHIT–RTIL/GCE in 0.1 mol L^{-1} phosphate buffer solution was higher than that in other supporting electrolytes, such as Briton Robinson, acetate, citrate and ammonia buffer solutions. Therefore 0.1 mol L^{-1} phosphate buffer solution was adopted as an optimum electrolyte for next experiments.

The effect of solution pH on the electrochemical response of the SWCNTs–CHIT–RTIL/GCE toward the simultaneous determination of 30 μmol L^{-1} ACT, 40 μmol L^{-1} URI and 70 μmol L^{-1} ASC was investigated using the DPV method. Variations of peak current with respect to pH of the electrolyte in the pH range from 3 to 10 are shown in Fig. 2. It can be seen that the anodic peak currents of ACT increase with raising the solution pH until it reaches 7. However at higher pH the ACT oxidation peak current starts to diminish. At low pH value CHIT has cationic ($-NH3^+$) form and by increasing pH it starts to deprotonate. The adsorption of ACT is probably enhanced at the low charged surface. The oxidation peaks of URI and ASC increase with pH up to pH 5 and then reach a plateau. These phenomena might be due to the interaction between the surface and the analyte charges, as well as pH dependent kinetics of electron transfers of URI and ASC. Therefore the pH value of 7, which is close to biological pH value, was chosen as an optimum solution pH for further experiments.

3.4. Linear dynamic range and detection limit of the method

The electrochemical response of simultaneous additions of ACT, URI and ASC into a 0.1 mol L^{-1} PBS pH 7 solution using SWCNTs–CHIT–RTIL/GCE is depicted in Fig. 3 and 4. Fig. 3 shows differential pulse voltammograms and corresponding calibration curves obtained at a SWCNTs–CHIT–RTIL/GCE from various concentrations of ACT, URI and ASC. For ACT a linear dynamic range from 2 to 200 μmol L^{-1}, and a detection limit of 0.11 μmol L^{-1} ($S/N = 3$) were obtained (Fig. 3 Inset:A). A linear relationship was found for URI in the range of 3–320 μmol L^{-1}, and a detection limit of 0.27 μmol L^{-1} (Fig. 3 Inset:B). A linear relationship was found for ASC in the range of 20 to 950 μmol L^{-1}, and a detection limit of 4.3 μmol L^{-1} (Fig. 3 Inset:C). The calibration equations for ACT, URI and ASC are as below:

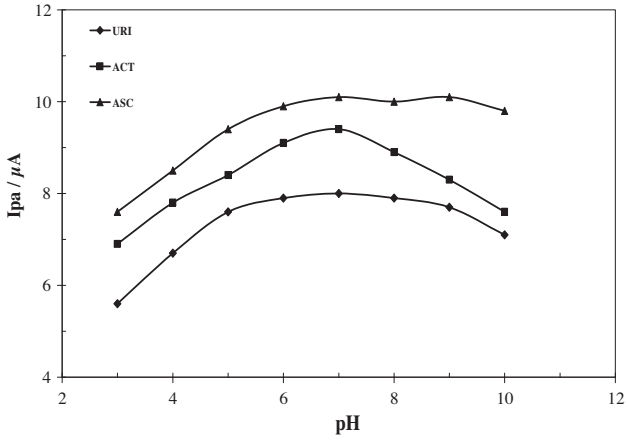

Figure 2 Effect of pH on the differential pulse voltammogram peak currents of oxidations of ACT, URI and ASC compounds at SWCNTs–CHIT–RTIL/GCE in phosphate buffer solutions. Concentrations: ACT: 30 μmol L^{-1}, URI: 40 μmol L^{-1} and ASC: 70 μmol L^{-1}.

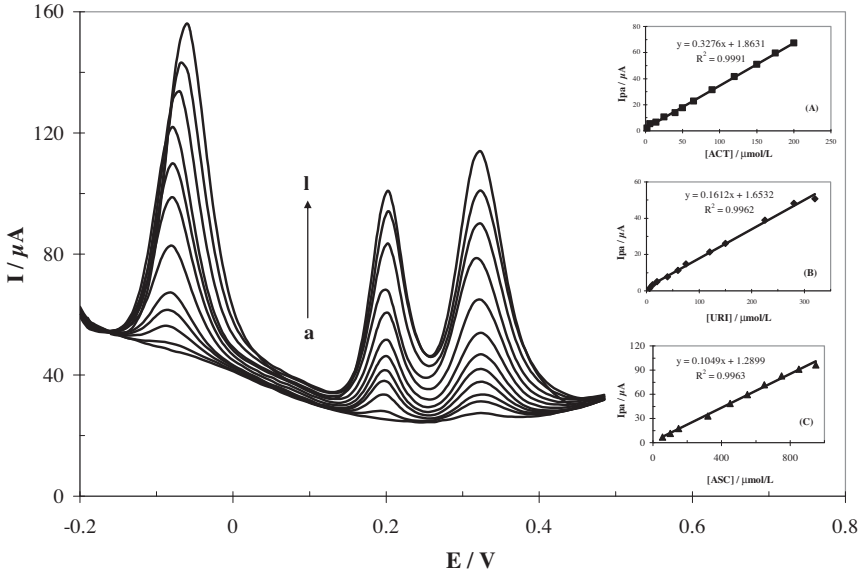

Figure 3 Differential pulse voltammograms for different concentrations of ACT, URI and ASC mixture as (a) 2 + 3 + 20, (b) 6 + 6 + 30, (c) 14 + 12 + 55, (d) 25 + 20 + 100, (e) 40 + 40 + 150, (f) 50 + 60 + 320, (g) 65 + 75 + 450, (h) 90 + 120 + 550, (i) 120 + 150 + 650, (j) 150 + 225 + 750, (k) 175 + 280 + 850 and (l) 200 + 320 + 950, respectively, in which the first value is the concentration of ACT, the second value is the concentration of URI and the third value is the concentration of ASC in μmol L^{-1}. (Insets: corresponding calibration curves).

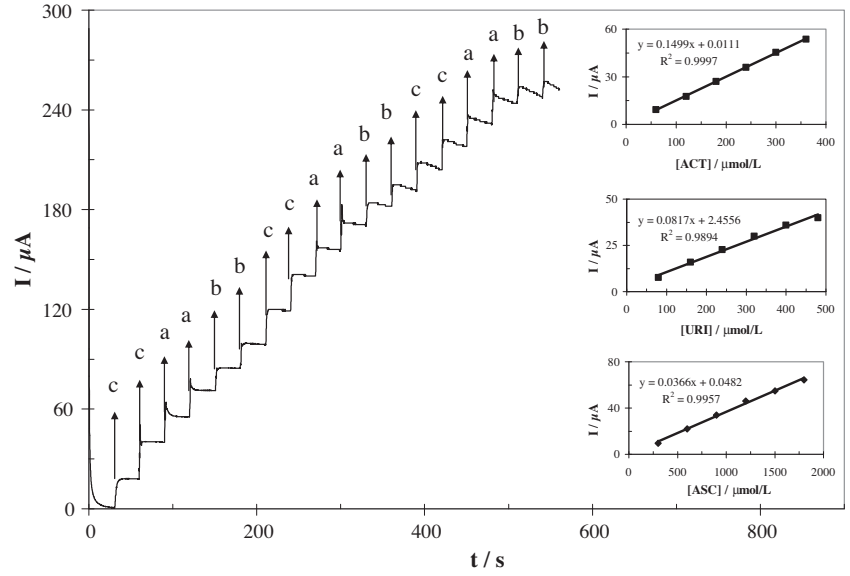

Figure 4 Amperometric response at rotating SWCNTs–CHIT–RTIL/GCE (rotating speed 2000 rpm) held at 0.45 V in PBS (pH 7) for determination of ACT, URI and ASC by successive additions of (a) 50 μmol L^{-1} ACT, (b) 80 μmol L^{-1} URI and (c) 300 μmol L^{-1} ASC (Insets: corresponding calibration curves).

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.3276c(\mu\mathrm{mol L}^{-1}) + 1.8631 \quad \text{ACT}$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.1612c(\mu\mathrm{mol L}^{-1}) + 1.6532 \quad \text{URI}$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.1049c(\mu\mathrm{mol L}^{-1}) + 1.2899 \quad \text{ASC}$$

Slopes of these calibration equations indicated that application of SWCNTs–CHIT–RTIL/GCE leads to high sensitivity in the simultaneous determination of ACT, URI and ASC.

Fig. 4 displays the chronoamperogram response of the rotated modified electrode (2000 rpm) with successive injection of ACT, URI and ASC at an applied potential of 0.45 V in PBS (pH 7). For ACT two linear ranges were obtained. The first linear dynamic range was from 10 μmol L^{-1} to 60 μmol L^{-1} and the second linear dynamic range was between 60 μmol L^{-1} and 360 μmol L^{-1}. A detection limit of 0.25 μmol L^{-1} (S/N = 3) was obtained. For URI two linear dynamic ranges were gained. The first linear relationship was in the range of 10 μmol L^{-1} to 80 μmol L^{-1} and the second linear relationship was in the range of 80 μmol L^{-1} to 480 μmol L^{-1}. A detection limit of 0.46 μmol L^{-1} was ob-

Table 1 Maximum tolerable concentrations of interfering species.

Interfering species	ACT[a]	URI[a]	ASC[a]
L-Dopa	400	350	600
dopamine	600	500	700
L-Alanin	1000	1050	1200
L-Glutamic acid	1300	1400	1800
L-Histidine	1600	1500	2000
L-Tyrosine	650	500	750
Aspartic acid	2000	1600	1800

[a] C_{int} refers to interfering compound concentration.

tained. For ASC two linear dynamic ranges were gained. The first linear relationship was in the range of 50 μmol L^{-1} to 300 μmol L^{-1} and the second linear relationship was in the range of 300 μmol L^{-1} to 1800 μmol L^{-1}. A detection limit of 1.03 μmol L^{-1} was obtained. The calibration equations for ACT, URI and ASC are as below:

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.1493c(\mu\mathrm{mol\,L^{-1}}) + 0.0003 \quad (R^2 = 0.9967)$$
$$10 \text{ to } 60\,\mu\mathrm{mol\,L^{-1}}\, ACT$$

$$\mathrm{Ip}(\mu A) = 0.1499c(\mu\mathrm{mol\,L^{-1}}) + 0.0111 \quad (R^2 = 0.9997)$$
$$60 \text{ to } 360\,\mu\mathrm{mol\,L^{-1}}\, ACT$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.0812c(\mu\mathrm{mol\,L^{-1}}) + 0.9721 \quad (R^2 = 0.9995)$$
$$10 \text{ to } 80\,\mu\mathrm{mol\,L^{-1}}\, URI$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.0817c(\mu\mathrm{mol\,L^{-1}}) + 2.4556 \quad (R^2 = 0.9894)$$
$$80 \text{ to } 380\,\mu\mathrm{mol\,L^{-1}}\, URI$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.0368\mathrm{c}(\mu\mathrm{mol\,L^{-1}}) + 0.0371 \quad (R^2 = 0.9998)$$
$$50 \text{ to } 300\,\mu\mathrm{mol\,L^{-1}}\, ASC$$

$$\mathrm{Ip}(\mu\mathrm{A}) = 0.0366c(\mu\mathrm{mol\,L^{-1}}) + 0.0482 \quad (R^2 = 0.9957)$$
$$300 \text{ to } 1800\,\mu\mathrm{mol\,L^{-1}}\, ASC$$

3.5. Repeatability and long-term stability of the electrode

Thus, the repeatability of the analytical signal has been studied. Indeed, the relative standard deviation (RSD) values of 0.92%, 0.76% and 0.96% for 100 μmol L^{-1} ACT, 100 μmol L^{-1} URI and 300 μmol L^{-1} ASC respectively in ten consecutive determinations have been obtained. Excellent repeatability of the modified electrode could be due to the anti antifouling property of chitosan (Tsai et al., 2007). Another advantage of the proposed modified electrode is that the resulting modified electrode is of a good long-term stability. Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of ACT, URI and ASC in solution or air. For example, determination of 100 μmol L^{-1} ACT, 100 μmol L^{-1} URI and 300 μmol L^{-1} ASC in 0.1 mol L^{-1} PBS (pH 7), when the modified electrode was subjected to an experiment every 30 min, after 24 h gave less than 8.3, 7.8 and 8.8% decrease in the voltammetric currents of ACT, URI and ASC, respectively. When the electrode was stored in the atmosphere for 7 days, the currents response of ACT, URI and ASC reduced less than 12.9, 12.7 and 13.9%, respectively when the electrode was used to analyze the solution containing 100 μmol L^{-1} ACT, 100 μmol L^{-1} URI and 300 μmol L^{-1} ASC. The relatively good stability of the electrode could be related to its high mechanical strength and high water stability of the CNTs-interspersed chitosan, which make it suitable for long-term electrochemical sensing applications.

3.6. Interference studies

This sensor is used for determination of ACT, URI and ASC in biological samples, so interfering effect of the presence species in biological samples must be investigated. The influences of common interfering species in the presence of 100 μmol L^{-1} ACT, 100 μmol L^{-1} URI and 300 μmol L^{-1} ASC under optimum conditions were investigated. The results showed interfering species did not significantly influence the peak currents for ACT, URI and ASC. The tolerance limit was defined as the concentrations which give an error of $\leqslant$10% on the determination of ACT, URI and ASC compounds. The data in the brackets are concentrations in μmol L^{-1} of the interfering spe-

Table 2 Determination of ACT, URI and ASC in human serum with SWCNTs–CHIT–RTIL/GCE.

Analyte	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	R.S.D. (%)	Recovery (%)
ACT	0.0	0.0		
	10.0	9.8	1.9	98.0
	20.0	19.5	1.5	97.5
	30.0	30.7	1.3	102.3
URI	0.0	6.2		
	20.0	26.3	1.8	100.5
	40.0	45.6	1.4	98.5
	60.0	65.0	1.1	98.0
ASC	0.0	0.0		
	100.0	99.1	2.4	99.1
	200.0	195.3	1.9	97.6
	300.0	289.6	1.6	96.5

[a] Average of five determinations at optimum conditions.

Table 3 Determination of ACT, URI and ASC in urine sample with SWCNTs–CHIT–RTIL/GCE.

Analyte	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	R.S.D. (%)	Recovery (%)
ACT	0.0	0.0		
	10.0	10.2	1.8	102.0
	20.0	19.5	1.4	97.5
	30.0	28.9	1.1	96.3
URI	0.0	43.8		
	20.0	64.4	2.5	103.0
	40.0	84.3	1.9	101.2
	60.0	102.8	1.2	98.3
ASC	0.0	0.0		
	100.0	98.7	2.2	98.7
	200.0	193.8	1.6	96.9
	300.0	297.2	1.3	99.1

[a] Average of five determinations at optimum conditions.

cies)Table 1(. The data show that the proposed method is free from interferences of the most common interfering agents.

3.7. Analytical applications

Applicability of the SWCNTs–CHIT–RTIL/GCE was examined for the determination of ACT, URI and ASC in human serum and human urine. The differential pulse voltammograms were obtained by spiking appropriate samples in the prepared real solutions using SWCNTs–CHIT–RTIL/GCE at optimum conditions as described earlier. The concentrations were obtained by applying calibration plot. The results are shown in Tables 2 and 3. The recoveries were acceptable, showing that the proposed methods could be efficiently used for the determination of trace amounts of these compounds in biological systems.

4. Conclusion

In this paper we introduced a sensor based on a single -walled carbon nanotube/chitosan/room temperature ionic liquid (SWCNTs–CHIT–RTIL) modified glassy carbon composite electrode. The SWCNTs–CHIT–RTIL composite can increase anodic peak currents of ACT, URI and ASC compounds with respect to currents obtained from a GCE. In addition the anodic peak currents for ACT and URI are well separated in comparison with those from the GCE. The results indicated that application of SWCNTs–CHIT–RTIL/GCE leads to high sensitivity and selectivity in the simultaneous determination of ACT, URI and ASC. The electrode showed high stability in repetitive experiments due to high water stability and high mechanical strength of the SWCNT-CHIT nanocomposite. The effects of potential interfering ions were studied, and it was found that the proposed procedure is free from interferences of most common interfering compounds. The simple fabrication procedure, high speed of use, reproducibility, high stability, wide linear dynamic range, low detection limit, and high sensitivity, suggest that the proposed sensor is an attractive candidate for practical applications.

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